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Enhanced bioavailability of cinnarizine nanosuspensions by particle size engineering: Optimization and physicochemical investigations

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ABSTRACT

Cinnarizine (CIN), a poorly soluble drug with erratic bioavailability due to pH dependent solubility has limited advantage to formulate oral solid dosage forms in subject having low gastric acidity. In present study precipitation-ultrasonication was used to fabricate nanosuspensions of cinnarizine stabilized by Poly vinyl alcohol (PVA) to enhance the bioavailability. We investigated the effects of PVA concentration (X_1) and solvent to antisolvent ratio (X_2) on the quality attributes like mean particle size (Y_1); % drug content (Y_2); and time required to 90% drug release (Y_3) via 3^2 factorial design. The morphology of nanosuspensions was found almost spherical by SEM observation. DSC and FT-IR studies revealed lack of significant interactions between CIN and PVA. Nanosuspensions of mean particle size 621.08 nm was achieved. The dissolution rate obtained from all formulations were markedly higher than pure CIN. Response surface methodology and optimized polynomial equations were used to select the optimal formulation i.e. 0.2% *W/V* of X_1 and 1:42 of X_2 to get the desired response Y_1; 636.78 nm, Y_2; 95.24% and Y_3; 7.09min that were in reasonable agreement with the observed value. The in-vivo study in rat demonstrated that C_{max} and $AUC_{0\rightarrow12}$ values of nanosuspension were approximately 2.8-fold and 2.7-fold greater than that of reference preparation respectively.

1. Introduction

A majority of the new medicinal entities going through different drug discovery program are not sufficiently efficient and only one drug out of every 5000–10,000 molecules get introduction into market where as other fails during the development pipeline. The drug entity thus discovered with appropriate biological activity frequently causes major challenges during pre-clinical formulation development [1]. The major cause is attributed to poor aqueous solubility, poor penetration and the properties of manufacturing variables during the development of a dosage form. For prompt bio-absorption, a drug candidate must have optimum hydrophilic and lipophilic balance as solubility in the GI fluid and permeability through biological membrane are two major determinants of bioavailability and therapeutic efficacy. It has been reported that major portion of recently developed drug candidates shows poor aqueous solubility [2] and thus belong to the classes II and IV of the Biopharmaceutical Classification System (BCS) [3].

Many approaches have been developed to overcome the aqueous solubility of very slightly water-soluble drug candidates. Drug nanonization have gaining importance in recent time as it is highly beneficial, particularly for low potency drugs requiring high doses and drugs for which oral route is the most convenient mode of administration [4]. Nanosuspension is the submicron drug particle stabilized by suitable polymer and/or surfactants [5]. 'Bottom up' and 'top down' are two approaches to produce nanosuspension. Top down approaches such as high pressure homogenization [7] and media milling [8] based on disintegration approach from large particle, microparticles to nanoparticles and bottom up techniques include microprecipitation, microemulsion, melt emulsification and so on, based on assembling method from molecules to nano sized particle to obtain stable nanosuspension [9].

Antisolvent precipitation is an effective and simple technique used to get stable nanosuspension of nitrendipine as described in literature [10]. In this method the drug is dissolved in a suitable solvent mixture which is then incorporated in to a pre-cooled anti-solvent solution containing stabilizer. A number of stabilizers in aqueous solution show a tremendous result in stabilizing the formulation such as HPMC & MC [11], povidone [12], and PVA [8].

Cinnarizine (CIN), (E)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl) piparizine, is a cerebral blood flow improver. It is widely used orally for the treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms with slow calcium channel blocking activity. It is also used for the control of nausea and vomiting

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[13,14]. CIN belongs to Class II drug in pharmaceutical Classification System (BCS) with low aqueous solubility. It is mainly absorbed in the upper gastrointestinal tract and has a short half-life of 4 h [15,16]. An important problem associated in obtaining solid oral dosage forms for this drug has been its poor aqueous solubility and wettability. Consequently, administration of CIN as a tablet or capsule results low and erratic oral bioavailability [17]. CIN shows a pH dependent absorption profile among the human volunteers and the bioavailability is a function of amount of drug dissolved in stomach. The major factor determining the amount of drug to be dissolved in the stomach is gastric pH. So a pharmaceutical mean of increasing the amount of the drug dissolved in stomach in the subject having low gastric acidity should be developed in order to overcome the pH dependent bioavailability of CIN in such patients [18].

Experimental tools such as factorial designs can be used to analyze and understand the process variables and finding out the appropriate combination of these to obtain a product with desired attributes. All factors were studied in all possible combinations with minimum experimentation and time to estimate the influence of individual process variables. This optimization technique involves the generation of model equations for the investigated responses over the experimental design to obtain the optimum formulation(s) [19].

To develop and optimize nanosuspensions of cinnarizine (NCIN), precipitation ultrasonication method had been employed. The effect of process variables i.e., stabilizer concentration in antisolvent (X_1) and solvent- antisolvent ratio (X_2) (two independent factors) on mean particle size (Y_1), % drug content (Y_2) and time required to 90% of drug release (Y_3; t90) (three dependent variables) were systematically investigated through response surface methodology (RSM) via 3^2 factorial design. The work involves assessment of in-vitro dissolution and in-vivo bioavailability ensuring the reproducibility of the investigation and eradicates solubility related bio-absorption of CIN.

2. Materials and methods

2.1. Materials

Cinnarizine was obtained as a gift sample from Dr. Reddy's Laboratories Ltd. (Hyderabad, Andhra Pradesh, India). Poly Vinyl Alcohol (PVA, 25 to 32 cp) and Polyethylene Glycol 200 (PEG 200) of analytical grade were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India. Acetone was procured from Merck Ltd., Mumbai, India.

2.2. Methods

2.2.1. Preparation of cinnarizine nanosuspensions

Nanosuspensions of cinnarizine (NCIN) were prepared by controlled precipitation-ultrasonication method. Cinnarizine (25 mg) was dissolved in 1 mL solvent mixture of acetone and PEG 200 (1:1, *V/V*) to obtained drug concentration of 25 mg/mL. Antisolvents containing different percentage of PVA (0.1%, 0.2%, 0.3%, *W/V*) were prepared in distilled water. The solvent- antisolvent ratios were taken as 1:20, 1:40 and 1:60 for the study. Both solutions were filtered through 0.45 μm filter (Nylon 66 membrane filter). Antisolvent solution was placed in a bath ultrasonicator (Sonica, Spincotech Pvt. Ltd. 2200 MH, 305 W, Soltech Srl Milano, Italy) and the water inside the ultrasonicator was cooled to 3 °C. The organic drug solution was quickly incorporated to the later with the help of a 22 gauze syringe after the application of ultrasonic wave. Ultrasonication was applied for a time period of 15 min for all nine formulations (Table 1). The obtained nanosuspensions were subjected to cooling ultracentrifugation (C-24 BL, Remi, Mumbai, India) at 20,000 rpm for 40 min. The supernatant was discarded and replaced by same quantity of fresh antisolvent. The solid residue was redispersed by sonication.

Table 1
3^2 full factorial design with observed response values of cinnarizine nanosuspensions.

Batch code	PVA (%*W/V*)	Antisolvent volume (mL)	Mean particle size (nm)	Average drug content (%)	Average time to 90% drug release (mins)	Z.P. (mV)
NCIN-1	0.1(−1)	20(−1)	1850.56	96.4	17.88	−22.17
NCIN-2	0.2(0)	20(−1)	912.74	96.57	10.09	−22.84
NCIN-3	0.3(1)	20(−1)	1250.54	98.89	13.19	−25.72
NCIN-4	0.1(−1)	40(0)	1550.04	94.70	15.91	−21.81
NCIN-5	0.2(0)	40(0)	621.08	95.27	6.36	−22.43
NCIN-6	0.3(1)	40(0)	872.21	97.45	8.67	−26.15
NCIN-7	0.1(−1)	60(1)	2013.91	92.84	19.75	−21.17
NCIN-8	0.2(0)	60(1)	1274.29	94.39	14.01	−23.05
NCIN-9	0.3(1)	60(1)	1720.13	96.71	16.83	−24.52

NCIN, cinnarizine nanosuspensions; PVA, poly vinyl alcohol; Z.P, zeta potential.

2.2.2. Isolation of dried nanosuspensions

The solidified state is preferred as compared with aqueous nanosuspensions, because aggregation and other instability factors are significantly decreased. Formulations were filled in flint colored vials with rubber stoppers and frozen using deep freezer at −75 °C for 24 h. These frozen semisolids were freeze-dried using lyophilizer (Virtis Benchtop, Bombay, India) at a vacuum degree of 200 Pa for 36 h to produce free flowing dry powder.

2.2.3. Characterization of cinnarizine nanosuspensions

2.2.3.1. Mean particle size, zeta-potential and particle morphology. Dynamic light scattering zetasizer (Zetasizer Nano ZS 90, Malvern Ltd., UK) was used to measure the particle size and zeta-potential. To assess the short term and accelerated stability, the particle size of nanosuspensions were analyzed after a short period and long period storage. The morphology was studied using scanning electron micrography (JEOL, JSM-6390, Tokyo, Japan). Images of platinum coated samples were captured using secondary detector. Examination of surface was performed at 3000×, 10,000× and 20,000× magnifications.

2.2.3.2. Differential scanning calorimetry. CIN pure drug, physical mixture of CIN and PVA, and freeze dried NCIN were analyzed by differential scanning calorimetry (DSC-60, Shimadzu Co., Japan) at a heating rate of 10 °C/min from 30 to 300 °C to study the phase transition. This study was done under dry nitrogen atmosphere using Al_2O_3 as a reference.

2.2.3.3. Fourier transform infrared spectroscopy (FT-IR). The molecular structures of the samples were recorded through FT-IR spectra using a FT-IR spectrometer (Shimadzu Corporation, Japan). Pure drug, PVA and nanosuspensions approximately of 2 mg were mixed with 100 mg of potassium bromide separately for preparing KBr disc. The IR spectra were obtained in KBr disc through scanning at a resolution of 2 cm^{-1} from 4500 to 400 cm^{-1}.

2.2.4. Drug content study

Drug content of the prepared nanosuspensions and all batches of nanosuspensions were determined by dissolving 10 mg of powder in 50 mL of hydrochloric acid solution (pH 3). The contents of CIN in sample were analyzed by double beam UV spectrophotometer (Model UV-1700, Shimadzu, Japan) at λ_{max} 253.5 nm. The drug samples were covered with the black color cotton cloth during the study to protect the solution from light. The procedure was performed in triplicate and the averages were calculated.

2.2.5. Dissolution study

In vitro drug release study of raw CIN powder and nanosuspensions was carried out in 900 mL of simulated gastric fluid medium (pH 1.2)